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Characteristics and Prognostic Implications of Rapidly Progressive Lethal Melanomas

MASTERARBEIT

zur Erlangung des akademischen Grades
Master of Medicine (M Med)
der Medizinischen Fakultät der Universität Zürich

vorgelegt von
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2017

Index

1. Abstract

Background

The incidence of melanoma is increasing worldwide. Recently, new treatment modalities changed the era in metastatic melanoma patients. However, there is still a group of patients who suffer from rapidly progressive disease and die shortly after being diagnosed with stage IV metastatic melanoma. Whether there are implications in genetic changes, clinical decision making or clinic-pathological characteristics in this group has to be elucidated.

Objectives

The aim of this master thesis is to analyze the group of patients with rapidly progressive lethal melanoma and to investigate their clinical characteristics and prognostic implications.

Materials and Methods

In this retrospective study, we analyzed 442 stage IV melanoma patients who have been treated at the Department of Dermatology, University Hospital Zurich from January 2008 until December 2014. The required information was gathered from the clinical electronic patient database (KISIM). 23 patients who died within the first 3 months, after being diagnosed with stage IV disease, formed our study group. A control group is composed also of advanced melanoma patients who have the best overall survival during this period.

Results

There is no significant difference in mutation status and characteristics of the primary melanoma between the study group and the control group. The negative prognostic factors seem to be elevated LDH and S100 levels, the male gender as well as bone, brain and liver metastasis.

Conclusion

We identified a patient group with rapidly progressive melanoma. We could confirm that patients with rapidly progressive melanoma (male, patients with elevated LDH/S100 levels, patients with brain/bone/liver metastasis) have high tumor volume. Further immunohistochemical and molecular investigation needs to be done.

2. Abbreviations

MM	Malignant Melanoma
IR	Incidence Rate
SR	Survival Rate
PFS	Progression Free Survival
OS	Overall Survival
CR	Complete Response
NF1	Neurofibromatosis 1
PD	Progressive Disease
USZ	University Hospital Zurich
AJCC	American Joint Committee on Cancer
MRT	Melanoma Registry Table
SG	Study Group
CG	Control Group
DFS	Disease Free Survival
SSM	Superficial Spreading Melanoma
NMM	Nodular Malignant Melanoma
ALM	Acral Lentiginous Melanoma
SNLB	Sentinel Lymph Node Biopsy
LDH	Lactate Dehydrogenase

3. Introduction

3.1. Melanoma Epidemiology

Malignant Melanoma (MM) is one of the most aggressive skin cancer, whose incidence is still increasing in many parts of the world, especially in light-skinned populations (1). This increase was observed over the last decades and is predicted to continue for at least two more (2). Switzerland has the highest incidence rate (IR) of newly developed MM per year in Europe (3). Although MM only represents 5% of all skin malignancies, it is responsible for the majority of lethal cases. Unlike its rising incidence, mortality has been stable since the 1980s in most parts of the world (2). This is mainly due to introduction of new treatment options, which resulted into survival rates (SR) improvement, new methods of early diagnostics as well as its diagnosis in very early stages (4, 5).

Due to its rising incidence, MM represents a major public health issue. Augmented UV exposure causing DNA damage is one well-studied factor for genetic changes resulting in melanoma formation and as of now the only that can be affected (6). This rise is not only a result of an increased recreational exposure to solar UV radiation, but as well as to indoor tanning activities (5, 7). Changes in style-clothing, contemporary tanning, expanded outdoor activities, ozone depletion and longevity contribute to this augmented exposure (8). Furthermore, international holidays are more accessible nowadays.

On the other hand, prevention is one of the major topics in the battle against cancer. According to questionnaires, 85% of Swiss adults use regular sun protection. Furthermore, women take sun protection more seriously than men do (89% versus 79%). In fact, the younger generations protect themselves more often then the older ones. The level of education as well as the income also have an influence: people with a higher education and income protect themselves better (6).

Since 2008 new treatment options changed the landscape of advanced melanoma and significantly improved not only progression free survival (PFS), but also overall survival (OS) (9, 10, 11, 12, 13, 14, 15, 16, 17). However, there is still a group of patients, who does not seem to profit from the new treatments. Over hundred BRAF

mutated patients with rapid disease progression under treatment with vemurafenib (BRAF-inhibitor) alone or combined with cobimetinib (MEK-inhibitor) were recently identified (18). Genomic differences between metastatic melanoma tumors from patients with complete response (CR) to therapy versus patients with progressive disease (PD) were suggested according to a recent data exploratory analysis. Patients with CR presented higher pre-existing immune response profile with enrichment of CD8 T effector cells, cytolytic T-cells, antigen presentation and NK cells, whereas patients with PD expressed more keratin genes (18).

3.2. Pathogenesis of Melanoma

In the last few years and with the introduction of next generations sequencing, a vast number of melanoma “driver” genes was identified (19).

Approximately 80% of melanoma patients have genetic alterations in oncogenes and tumor suppressor genes, which regulate the mitogen activated protein kinase pathway (MAPK). This leads to a constitutive signaling trough RAS-RAF-MEK-ERK (Fig. 1) and eventually in alteration of cell proliferation and senescence (20) (21). The most common mutation is BRAF, which is harbored by approximately 50% of all melanomas, followed by NRAS, which is also mutated in nearly 30% of all melanoma patients (19, 22). Mutations in other pathways as WNT or PI3K are less common but also very important.

Krauthammer et al. additionally identified new important mutations in melanoma, including neurofibromatosis 1 (NF1), the third most common mutated gene in melanoma after BRAF and NRAS. NF1, a negative regulator of RAS, leading to a higher RAS activation when possessing an inactivating mutation (19). NF1 represents a key tumor suppressor, which is often lost in melanomas. The Cell Genome Atlas recently proposed a new genomic classification for cutaneous melanoma as: mutated BRAF, mutated NRAS, mutated NF1 and triple wild-type (23). This classification facilitates clinical decision-making based on targeted therapies.

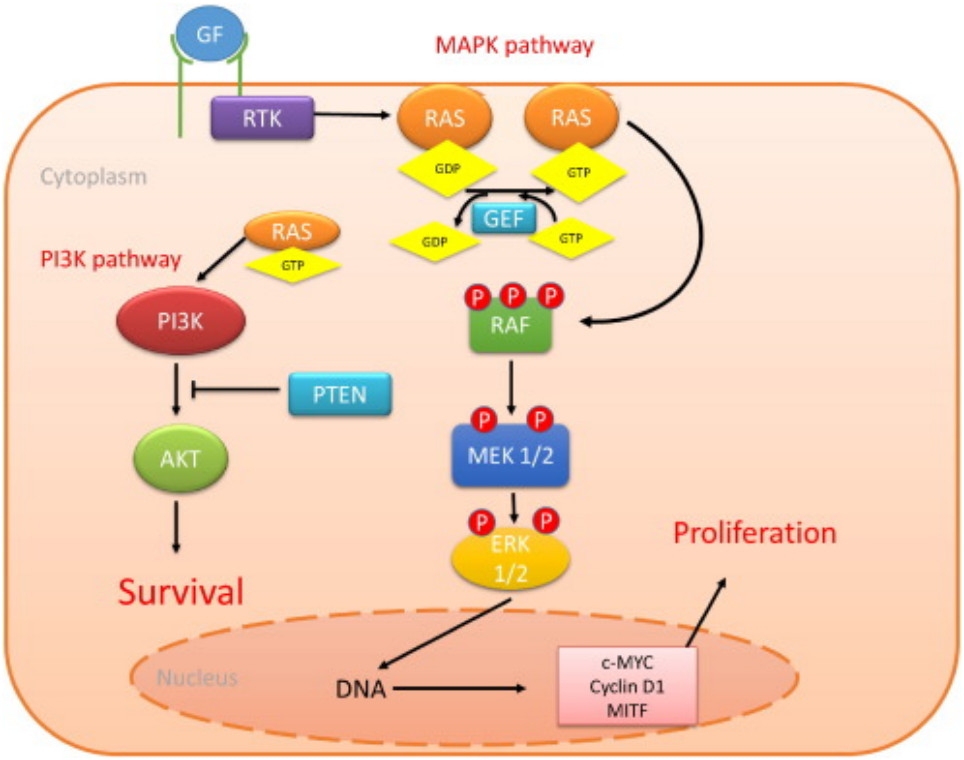

Fig. 1: MAPK- and PI3K-Pathway (21).

3.3. Metastatic Melanoma

As soon as the tumor cells break through the basal membrane, then the disease becomes invasive into deeper dermis and subcutaneous tissue. The dissemination can either be hematogenous or through the lymphatic vessels.

3.3.1. Loco Regional Metastasis

Loco regional metastasis comprise of local recurrences, locoregional lymph node metastasis, satellites metastasis (which appear inside a radius of 2cm from the primary tumor) as well as skin or subcutaneous metastasis, which occur along the lymph pathways between the primary tumor and the regional lymph nodes (in transit metastasis).

3.3.2. Distant Metastasis

In general, neoplastic development progresses through several stages as tumor initiation, progression, invasion and then metastasis (24). To perambulate those

steps, malignant cells need to acquire certain carcinogenic features such as unlimited proliferation, evasion of cell intrinsic and environmental restrictions, attraction of a blood supply and the capacity to detach and distance itself from the original location. These features are prerequisites for the tumor initiation and progression (25). Further genetic alterations have to occur before malignant cells become invasive, enter the circulation and evade the immune system to colonize other organs (25). The fact, that melanocytes are naturally resistant to ultraviolet light and reactive oxygen species, is one reason why melanoma cells are particularly resistant to killing (24).

Furthermore, it has been proposed that malignant melanoma cells have two gene expression signatures, which is either proliferative (upregulation of MITF, TYR and DCT) or invasive (upregulation of WNT5A, TGFβ and FGF2). The cells can switch from one signature to the other in response to microenvironmental changes (=phenotype switch). Recently, the SOX9-gene overexpression in malignant melanoma cells could be identified as an important gene in melanoma invasion (26).

When the tumor cells spread beyond the loco regional lymph nodes, then the disease is defined as distant.

3.4. Prognostic Factors

Several factors, designating as prognostic factors, which implicate the outcome of melanoma patients, have been investigated over the years. In the following subchapters the main ones are listed.

3.4.1. Patient-related

3.4.1.1. Gender

In a recently published Italian study (involving 3900 patients) Crocetti et al. showed that women who suffer from cutaneous melanoma have an improved 5-year disease specific survival compared to men (women = 87% vs. men = 80%, 95%-confidence interval: 86% – 89% and 78% – 82%, respectively). Survival did not seem to implicate with disease stages. The cause of this superiority remains still unknown

(27). Consistently, it has been suggested, that women have a better prognosis in every stage of the disease, due to lower risk of visceral metastasis and lymph node invasion (28). What could also contribute to a better survival is the postulation that women look better after their health and skin. On the other hand, another study showed that women have a higher risk to be diagnosed with melanoma (29).

3.4.1.2. Age

Until recently, age has been reported to be an independent prognostic factor for the outcome of melanoma patients (30, 31, 32). However, in 2013 Balch et al. reported that the patient's age is a powerful and highly significant predictor of survival. They came to this result even after accounting for adverse prognostic features, e.g. anatomic site of the primary melanoma and patient's gender. Furthermore, this study suggested that younger patients (<20 years) have a significant better survival than all other age groups ($p < 0.0001$). This seems paradoxical due to the fact that these patients had a primary tumor with slightly more aggressive characteristics and a higher incidence of sentinel lymph node micrometastasis than all other age groups. The lowest survival is observed in patients over 70 years ($p < 0.0001$) (33). In a large study with more than 17'000 patients, every 10-year increase in age was associated with a decrease in 5- and also 10-year survival rates (32)

3.4.1.3. Localization

Cutaneous melanoma appears more frequently on the back and limbs (especially the lower limb in women), followed by the head and neck, and then the anterior trunk (34, 35). The typical sites for the primary melanoma in younger patients are trunk and limbs. In older patients, it mostly occurs on sun exposed skin areas as the head and neck. Patients who develop melanomas on the trunk have markedly higher nevus count, which is known to be another risk factor for melanoma, than those whose melanomas arise on sun-exposed sites (36).

A correlation between anatomic location and prognosis has been postulated. Melanomas of the head, neck and trunk, have a poorer prognosis compared to melanomas on the extremities (37, 38, 39).

3.4.2. Histology

3.4.2.1. Thickness

The thickness of the primary tumor, known as Breslow index, and measured in millimeters from the granular layer to the deepest identifiable tumor manifestation, has significant prognostic implications (40). It is the most powerful independent prognostic variable in localized melanoma, followed by ulceration. In a study of over 11'000 patients Balch et al. showed a highly significant decrease in 5- and 10-year survival rates as tumor thickness increased (41). The AJCC melanoma staging stratified the thickness in four categories for the T-staging: ≤1.0 mm, 1.01-2.0 mm, 2.01-4.0 mm, >4 mm (32).

3.4.2.2. Ulceration

Already in 1953, Allen & Spitz described ulceration, defined as the absence of an intact epidermis, as another adverse prognostic factor (42). Balch et al. illustrated in 1980 that patients with ulcerated melanoma had a poorer survival in comparison to non-ulcerated melanoma, even though other factors, such as gender and age, had been accounted for (43). Ulceration correlates highly with tumor thickness. It has been shown, that with the increased tumor thickness, also the incidence of ulceration rose (6-12.5% for thin, 63-72.5% for thick melanomas, $p < 0.0001$) (32, 43). However, in contrast to the thickness, ulceration remains an important prognostic factor even if nodal metastasis appear (stage III). It is the only feature of the primary tumor that indicates a adverse outcome in stage III (32). They came to the conclusion that "ulceration reflects a more aggressive and infiltrative character of melanoma than non-ulcerative lesions" (31). The incorporation of ulceration in the AJCC melanoma staging system took place in 2001 (32).

3.4.3. Laboratory Parameters

S100

S100 is a protein, which plays a central role in the signal transduction of the cell cycle regulation. There are two subtypes of the S100 protein: S100A and S100B. S100B is only expressed in cells of the nervous system, cartilage cells, adipocytes and cells of melanocytic origin (44). Initial, it has been used as a serum marker for acute brain

damage. About 20 years ago S100B was first described as a serum marker for malignant melanoma (45). Nowadays S100B-diagnostics is widely accepted as the main tumor marker in malignant melanoma used in the clinic (46). The concentration of S100B in the serum is strongly depending on the tumor burden. When the tumor burden is high, the S100B level in the serum rises. S100B is useful in the following situations:

- Early detection of disease progression in tumor free patients → an elevation of S100 in comparison to the initial value indicates a new activity of the tumor.
- Treatment monitoring and response → a decline of S100 is associated with a tumor mass reduction.
- Estimation of the OS in patients with distant metastasis → patients with a value clearly over the reference range, have a poorer OS than patients with average values (46).

LDH

LDH (lactate dehydrogenase) is a ubiquitous cytoplasmatic enzyme in the human body and represents the key enzyme of the glycolysis as it catalyzes the turnover from lactate to pyruvate. As LDH is expressed in almost every human cell, its blood level increases with tissue damages of all kinds. An elevation of LDH can happen in the following diseases:

- Tissue damage or hemolysis (e.g. trauma)
- Heart muscle or liver damage (e.g. myocardial infarction)
- Myopathy (e.g. myositis)
- Malignant neoplasia (46)

In solid tumors, including malignant melanoma, elevated LDH levels are associated with an impaired survival (47). Elevated serum levels of LDH strongly correlate with metastatic disease. In 2001, LDH was the first laboratory parameter being incorporated in the leading system of tumor staging in malignant melanoma, AJCC (American Joint Committee on Cancer) (32). In the current AJCC staging, patients with distant metastasis, independent of its localization, and additional elevated LDH are listed as M1c, as it is known that the prognosis of these patients is poorer (41).

In a current published study of 617 advanced melanoma patients (from randomized phase III clinical trials), treated with a combination of BRAF- (dabrafenib) and MEK inhibitor (trametinib), were retrospectively analyzed in terms of predictive factors for clinical outcome.
It has been showed that patients with normal LDH levels had a substantial better 1- and 2-year OS and PFS in comparison to patients with LDH levels at least two times the upper limit of normal (48). However it is not known, whether elevated LDH levels are marker of aggressive disease or if it has a direct causative role in response to treatment or tumor growth (48).

Recently, Frauchiger et al. suggested a significant negative impact of elevated LDH levels on OS in both BRAF mutated and wild type patients. With this findings they could confirm LDH as an BRAF independent marker for survival in advanced melanoma in their study (49).

3.4.4. Pattern of Metastasis

Overall, primary melanomas mostly metastasize to the regional lymph nodes, but approximately one third of them directly presents with metastasis at distant sites (24, 50). The most common sites of distant metastasis are lymph node, skin, lungs, liver, brain and bone. Metastasis of the lungs, liver and brain are the most common causes of death in advanced MM patients (51, 52). Meier et al. showed that the localization of primary tumor is a strongly predictor of where metastasis would develop, based on a retrospective clinical trial of 466 patients. They suggested, that patients with primaries at the extremities or on the trunk most probable develop satellite or in-transit metastasis, whereas tumors of the head and neck can develop in-transit, lymph node or directly present with distant metastasis (50).

Furthermore, in another study with more than 1’100 stage IV melanoma patients, the site of distant metastasis was the only significant prognostic feature for clinical outcome. It was shown that non-visceral metastasis have the best survival, followed by lung metastasis and then all other visceral metastasis (32).

In a recent study, no difference in metastatic spread between BRAF mutant and BRAF wild type could be shown (49).

3.5. Formulation of a Question

In this retrospective study, we aimed to investigate the subgroup of advanced melanoma patients who suffered from rapidly progressive disease and died within 3 months after stage IV diagnosis. We analyzed possible clinico-pathological factors, which might indicate a rapid disease progression in this minority of patients.

Hypotheses:

1) Melanomas, which metastasize hematogenous (stadium N0M1c), are more aggressive than lymphatic metastasizing melanoma.
2) A high number of afflicted organs indicate an unfavorable outcome.
3) Bone, brain or liver metastasis serve as poor prognostic factors.
4) Lymph node and lung metastasis have a better prognosis than other visceral metastasis (M1a/M1b vs. M1c).

4. Materials and Methods

4.1. Patient Selection and Data Acquisition

In this retrospective study we evaluated all stage IV metastatic melanoma patients being treated at the Dermatology Department of the University Hospital Zurich (USZ) during the period January 2008 - December 2014. Stage IV disease was defined according to the current American Joint Committee on Cancer (AJCC) staging system. Data were collected by reviewing patient's electronic medical files in our clinical database KISIM. All patients who fulfilled the inclusion criteria were registered in the Melanoma Registry Table (MRT). At the time of analysis, the MRT contained 422 patients, 322 of them were dead and 120 still alive. Treatment after the first distant metastasis as well as epidemiological, clinic-pathological, laboratory and molecular parameters for each patient were collected.

Our cohort was divided in two groups according to the OS (Fig. 2). OS was defined as the time (in months) from diagnosis of the first distant metastasis to death/last follow up. Cut off of follow up data was defined as in September of 2016. The study group (SG) includes metastatic melanoma patients with an OS of 3 or less months and contained 23 patients. The control group (CG) contains the 50 patients with the best SR (range: 38.4 – 173.5 months).

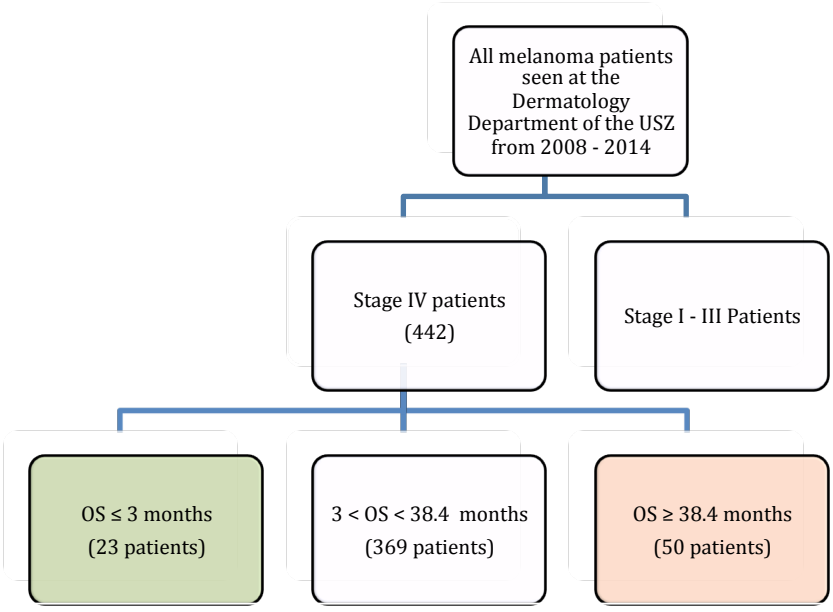

Fig. 2: Green = Study Group; Orange = Control Group

Furthermore, disease free survival (DFS) is defined as the length of time in months from the diagnosis of the primary tumor to first distant metastasis. For the T-staging, the Breslow index is divided in four subgroups: T1 = 0-1.0mm, T2 = 1.01-2.0 mm, T3 = 2.01-4.0 mm, T4 > 4.0 mm. Normal LDH and S100 levels were defined as lower or equal to the reference cut-offs of 480 U/l and 0.2 ug/l, respectively, as defined by the normal ranges of the local laboratories. We divided the number of afflicted organs in two subgroups: >2 and ≤2.

At the time of data cut-off, one patient of the CG was still in stage IIIC disease. One patient of the SG died from an apoplexy (ACM left).

4.2. Definition of Baseline and Endpoints

Primary endpoint of this study was to identify the patient group with rapidly progressive lethal melanoma and to determine differences in clinical characteristics and its prognostic implications. Secondary endpoints included differences in time to first distant metastasis between our SG and CG, and differences in OS with brain, liver or bone metastasis versus without between the SG and CG.

4.3. Statistical Analysis

Descriptive statistics are listed as percentages of total for categorical variables and ordinal variables and median for continuous variables. OS was estimated with the Kaplan-Meier survival curves. Univariate logistic regression was performed on all clinically relevant factors between SG and CG. A p-value less than 0.05 was deemed as statistically significant. Precise clinical information was only available in 47 patients in our CG and these are the ones that were used for the statistical survival analyses.

4.4. Ethics

Local ethics committee approved written informed consent for tissue storage including retrospective analysis with collection of clinical / laboratory / histological information (KEK-ZH-Nr. 647, 800).

5. Results

5.1. Patient Characteristics

For the MRT, 442 AJCC Stage IV melanoma patients who received systemic treatment for the disease from January 2008 until December 2014 were gathered. 170 (38.5%) were female, 272 (61.5%) were male. Median age at first diagnosis was 57 years (range 13.4 – 91.7 years). At the time of the last follow-up in September 2016, 120 (27.1%) patients were alive, 322 (72.9%) deceased.

For all patients, histopathologic information such as melanoma subtype, localization of primary tumor and tumor thickness were available. The presence of ulceration was obtainable in 36.2% of the patients. LDH- and S100-levels were known in 66.7% and in 67.2% of the patients respectively.

83 patients fulfilled the inclusion criteria for the study; from those 23 formed the SG and 50 the CG.

All the laboratory parameters as well as patient's characteristics are listed in Table 1.

	Study Group	Control Group
Age (Median)	60.8	52.2
Breslow		
0.01-1	3 (13%)	5 (10%)
1.01-2	3 (13%)	6 (12%)
2.01-4	7 (30.4%)	9 (18%)
>4mm	7 (30.4%)	13 (26%)
unknown	3 (13%)	17 (34%)
CNS Metastases		
no	20 (87%)	50 (100%)
yes	3 (13%)	0 (0%)
Bone Metastases		
No	9 (39.1%)	44 (88%)
yes	14 (60.9%)	6 (12%)
Liver Metastases		
No	7 (30.4%)	43 (86%)
yes	16 (69.6%)	7 (14%)
Number of Metastases		
1-5	0 (0%)	40 (80%)
6-15	1 (4.3%)	3 (6%)
>15	22 (95.6%)	7 (14%)
Number of afflicted organs		
≤ 2	7 (30.4%)	48 (96%)
> 2	16 (69.6%)	2 (4%)
N0M1c		
Yes	2 (8.7%)	2 (4%)
no	21 (91.3%)	48 (96%)
M-Stadium		
M1a/b	2 (8.7%)	29 (58%)
M1c	21 (91.3%)	20 (40%)
M0	0 (0%)	1 (2%)
LDH		
elevated	17 (73.9%)	1 (2%)
normal	6 (26.1%)	23 (46%)
unknown	0 (0%)	26 (52%)
S100		
elevated	20 (87%)	11 (22%)
normal	2 (8.7%)	18 (36%)
unknown	1 (4.3%)	21 (42%)
Mutation status		
BRAF mut	9 (39.1%)	27 (54%)
NRAs mut	1 (4.3%)	7 (14%)
Sex		
Female	7 (30.4%)	25 (50%)
Male	16 (69.6%)	25 (50%)
Therapy		
IT	4 (17.4%)	13 (26%)
TT	5 (21.5%)	12 (24%)
IT + TT	1 (4.3%)	18 (36%)
Chemo	9 (39.1%)	5 (10%)
Chemo + TT	2 (8.7%)	0 (0%)
Ulceration		
No	6 (26.1%)	9 (18%)
Yes	6 (26.1%)	7 (14%)
Unknown	11 (47.8%)	34 (68%)
Melanoma Type		
SSM	5 (21.7%)	8 (16%)
NMM	7 (30.4%)	10 (20%)
ALM	1 (4.3%)	2 (4%)
Desmoplastic	0 (0%)	1 (2%)
Mucosal	1 (4.3%)	2 (4%)
Choroidal	0 (0%)	2 (4%)
Amelanotic	0 (0%)	4 (8%)
Other*	1 (4.3%)	0 (0%)
Unknown	8 (34.7%)	21 (42%)
Localisation		
Acra	1 (4.3%)	2 (4%)
Lower Limb	9 (39.1%)	13 (26%)
Upper Limb	1 (4.3%)	6 (12%)
Trunk	5 (21.7%)	12 (24%)
Head/Neck	4 (17.4%)	7 (14%)
Sinunasal	1 (4.3%)	1 (2%)
Uveal	0 (0%)	2 (4%)
Other	1 (4.3%)	1 (2%)
unknown	1 (4.3%)	6 (12%)

Table 1: TT: targeted therapy, IT: immunotherapy, CNS: central nervous system, LDH: lactade dehydrogenase, SSM: superficial spreading melanoma, NMM: nodular melanoma, ALM: acrolentiginous melanoma, other: polypoid, ex naevo, not classified*

5.2. Study Group

Patient's characteristics and demographics

In the SG, 7 (30.4%) patients were female and 16 (69.6%) were male, median age at first diagnosis was 60.8 years (range 29 – 81.1 years).

Localization and type of primary melanoma

The most common localization of the primary melanoma was the lower limb (39.1%), followed by the trunk (21.7%) and then head/neck (17.4%). One patient had a sinonasal melanoma, while one had a melanoma of an unknown primary. The most common melanoma subtype was NMM (30.4%), followed by SSM (21.7%). One patient was diagnosed with ALM (4.3%) and another with mucosal melanoma. In 8 patients the melanoma type was unknown (34.8%).

Breslow-index and ulceration

The patient distribution was as follows: three (13%) patients were classified as T1, 3 (13%) as T2, 7 (30.4%) T3 and 7 (30.4%) as T4. In 3 (13%) patients the Breslow index was unknown. In approximately 50% of the patients the ulceration status was unknown. Six (26.1%) had a primary with ulceration, while 6 without.

Laboratory parameters

The LDH-level was elevated in 17 (73.9%) and was normal in 6 (26.1%) patients. The S100-level was elevated in 20 (87%). Two patients had normal S100 levels and of one the S100 was not available.

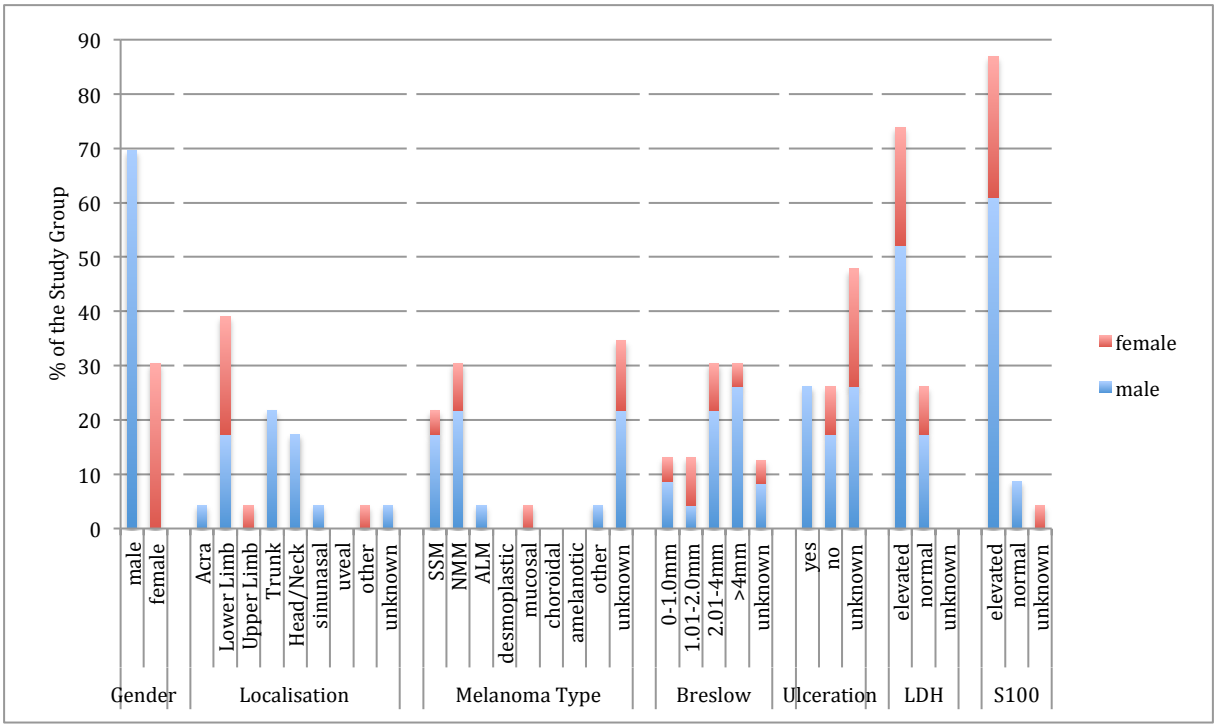

Fig. 3: Characteristics of the primary melanoma and laboratory parameters of the SG.

Mutation status of patient population

Nine (39.1%) patients harbored a BRAF mutation, 10 (43.4%) were BRAF-wild type and in 4 (17.4%) the BRAF mutation status was unknown. One (4.3%) patient harbored a NRAS mutation and 15 (65.2%) were NRAS-wild type. The NRAS mutation status was not known in 7 (30.4%) of the patients (see Fig. 4).

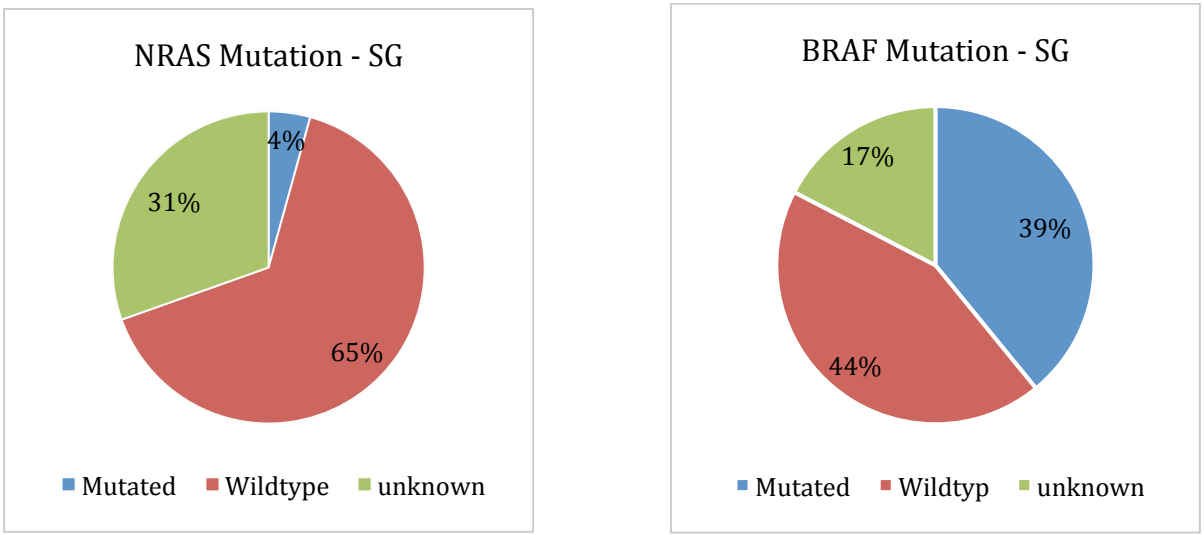

Fig. 4: Mutation status of the SG; left: NRAS mutation, right: BRAF mutation.

Disease free survival and overall survival

The mean DFS was 38.6, the median 9.1 months (range 0 – 235.6 months). The mean OS was 2.1, the median 2.4 months (range 0.5 – 3.0 months).

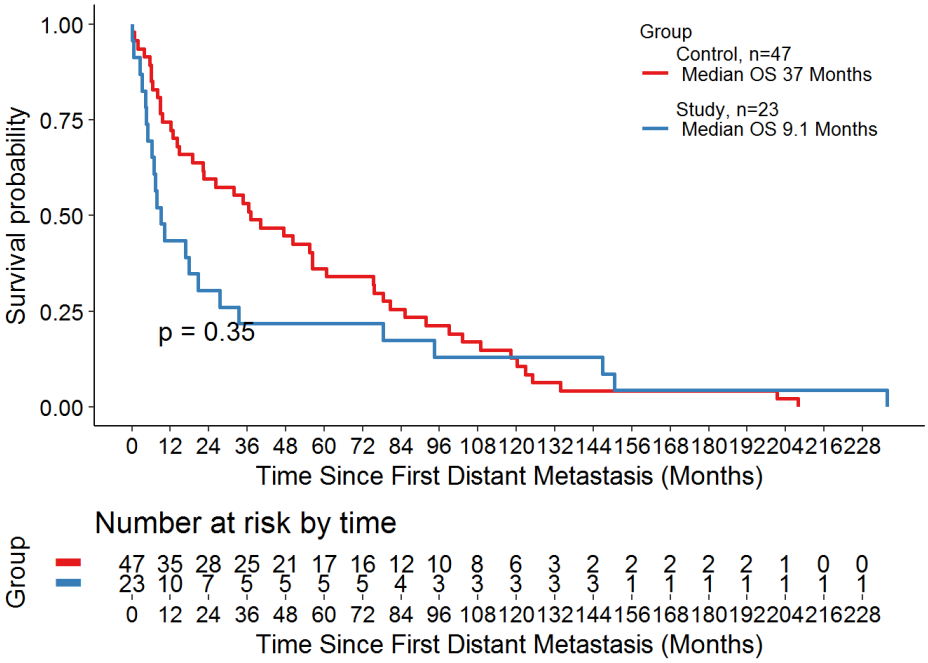

Fig. 5: DFS: Time from stage I to stage IV diagnosis in months.

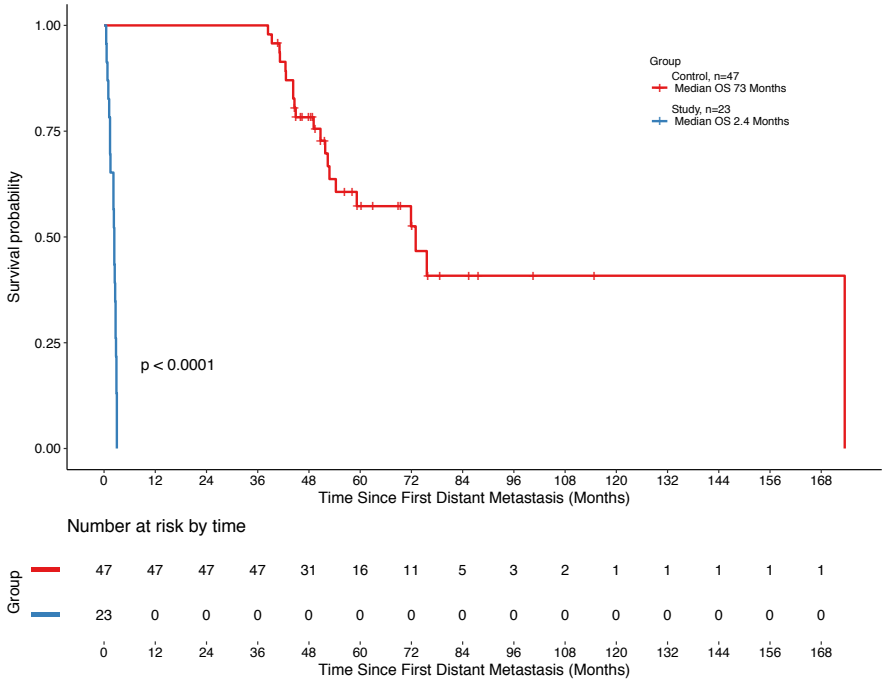

Fig. 6: Kaplan-Meier survival curve of SG and CG

Coefficient	OR	Lower 95% CI	Higher 95% CI	Pvalue
Age	1.05	1.01	1.11	0.0236
Female	0.304	0.121	0.674	0.00585
Male	0.667	0.348	1.24	0.209
T1	0.6	0.123	2.45	0.484
T2	0.5	0.106	1.9	0.327
T3	0.875	0.307	2.44	0.796
T4	0.538	0.202	1.31	0.187
BRAF Mutant NRAS WT	0.346	0.153	0.712	0.00609
BRAF WT NRAS Mutant	0.167	0.00882	0.976	0.0971
BRAF WT NRAS WT	0.667	0.223	1.85	0.442
BRAF WT NRAS unknown	0.6	0.123	2.45	0.484
Ulceration No	0.75	0.247	2.16	0.594
Ulceration Yes	0.857	0.276	2.58	0.782
LDH normal	0.286	0.105	0.666	0.0068
LDH elevated	17	3.49	306	0.0059
S100 normal	0.118	0.0187	0.41	0.0042
S100 elevated	1.82	0.887	3.93	0.111
Affected organs <= 2	0.133	0.051	0.289	3.55e−06
Affected organs > 2	8.5	2.44	53.6	0.0042
1–5 metastases	1.17e−09	1e−05	6.35e+50	0.994
6–15 metastases	0.25	0.0128	1.69	0.215
> 15 metastases	3.14	1.41	7.95	0.00832

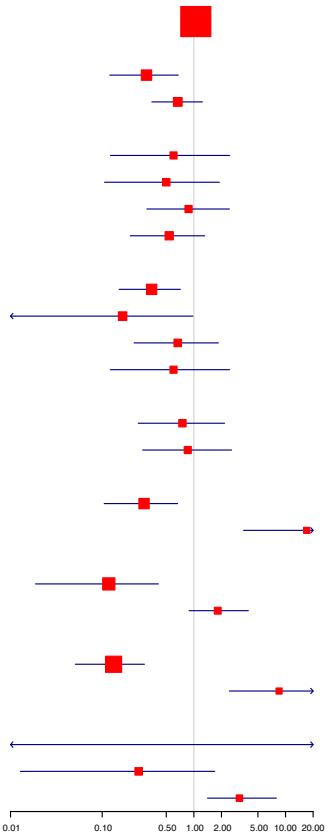

Fig. 7: Forest Plot

Distant metastasis

At stage IV disease, 14 (60.9%) patients were diagnosed with bone metastasis. On the other hand, liver metastasis were detected in 16 (69.6%) patients. The majority of the patients did not have brain metastasis (20 without versus 3 with). 21 (91.3%) patients were diagnosed with N1-3M1 melanoma (according to AJCC), while only 2 patients were diagnosed with N0M1. 16 (69.6%) patients had more than two afflicted organs at the time of stage IV disease, 7 (30.4%) had two or less afflicted organs. 2 (8.7%) patients had an M1a or M1b stadium compared to 21 (91.3%) with M1c.

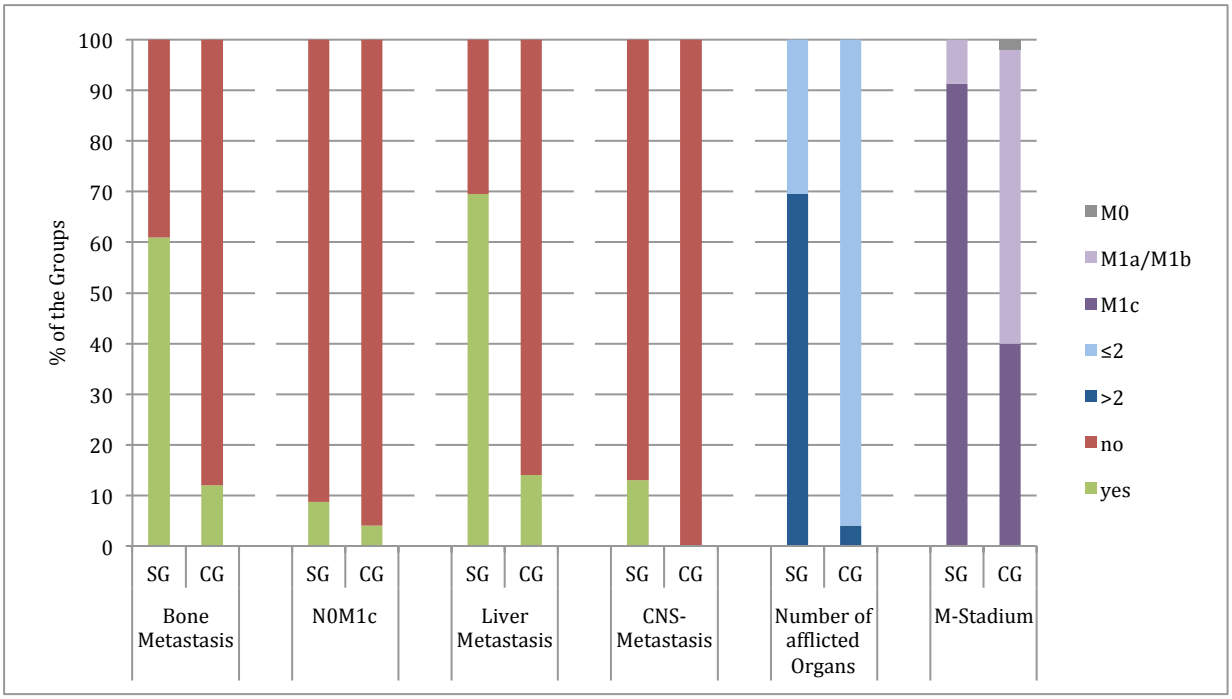

Fig. 8: Characteristics of distant metastasis when patients entered stage IV. Comparison of SG and CG.

Initial treatment

Nine (39.1%%) patients of the SG received chemotherapy as first treatment at time of stage IV disease. Five (21.7%) were treated with targeted therapy and four (17.4%) received immunotherapy. Two (8.7%) patients received chemotherapy combined with the multikinase inhibitor sorafenib (DTIC and sorafenib), one (4.3%) a combination of immunotherapy and targeted therapy, as at that time none of the new treatments were available. (One (4.3%) patient received chemotherapy in combination with darleukin (L19IL2, a human immunostimulatory antibody), and another one (4.3%) patient was treated with pasireotide (NCT01652547).

5.3. Control Group

Patient's characteristics and demographics

In the CG 25 (50%) were female and 25 (50%) were male, median age at first diagnosis was 52.2 years (range 18.9 – 72 years).

Localization and type of primary melanoma

The most common localization of the primary melanoma was the lower limb (26%), followed by the trunk (24%), head/neck area (14%) and the upper limb (12%). Two patients presented with an acral melanoma (4%), two with uveal and one with sinonasal. In 6 patients the localization was unknown (12%). The most common melanoma subtype was NMM (20%), followed by SSM (16%) and ALM (4%). Four patients were diagnosed with amelanotic (8%), two with mucosal (4%), two with choroidal (4%) and one with desmoplastic (2%) melanoma. In 21 patients the melanoma type was not classified (42%).

Breslow-index and ulceration

5 (10%) patients were classified as T1, 6 (12%) as T2, 9 (18%) T3 and 13 (26%) as T4. In 17 (34%) patients the Breslow index was unknown. In 7 (14%) patients the primary tumor carried ulceration, in 9 (18%) there was no ulceration noted. In 34 (68%) patients the ulceration status was unknown.

Laboratory parameters

The LDH-level was elevated in 1 (2%), was normal in 23 (46%) and unknown in 26 (52%) patients. The S100-level was elevated in 11 (22%), was normal in 18 (36%) and unknown in 21 (42%) patients.

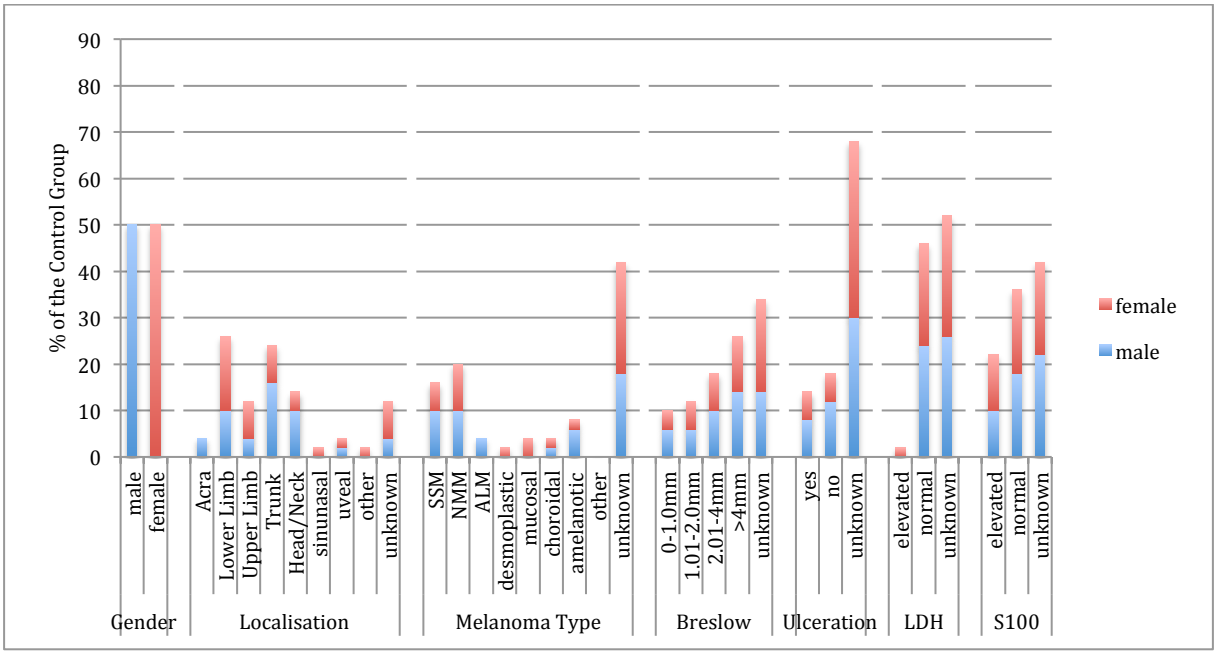

Fig. 9: Characteristics of the primary melanoma and laboratory parameters of the CG

Mutation status of patient population

27 (54%) patients harbored a BRAF mutation and 22 (44%) were BRAF-wild type. In one patient (2%) and in 7 (14%), the BRAF and NRAS status was unknown respectively. 7 (14%) had a NRAS mutation and 36 (72%) were NRAS-wild type.

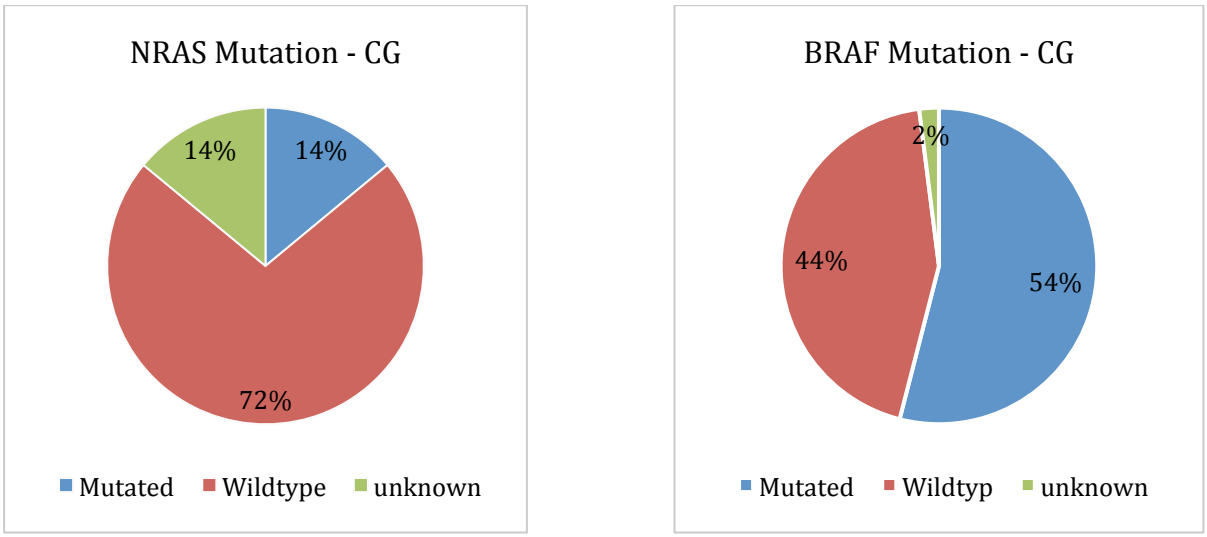

Fig. 10: Mutation status of the CG; left: NRAS mutation, right: BRAF mutation.

Disease free survival and overall survival

The mean DFS was 52.3, the median 37 months (range 0 – 207.9 months). The mean OS was 59.6, the median 73 months (range 38.4 – 173.5 months).

Distant metastasis (Fig. 8)

At stage IV disease, only 6 (12%) patients had bone metastasis. Liver metastasis were detected in only 7 (14%) patients, while 43 (86%) did not have any liver metastasis at this stage. None of the patients had brain metastasis at stage IV disease. AJCC stadium N1-3M1 was found in 48 patients (96%), N0M1 only in 2 patients (4%). 2 (4%) patients had more than two afflicted organs at the entering of stage IV, 48 (96%) had two or less afflicted organs. 29 (58%) patients had an M1a or M1b stadium when entering stage IV in comparison to 20 (40%) patients who were in stadium M1c, 1 (2%) patient was still in stadium M0 (stage IIIC).

Initial Treatment

Most of the patients of the SG were initially treated with a combination of immunotherapy and targeted therapy (36%). 13 patients were treated with

immunotherapy (26%) and 12 with targeted therapy (24%) alone. 5 patients were first treated with chemotherapy (10%). One patient was treated within the NY-ESO-1 (NCT01213472) another with chemotherapy (dacarbazine) and darleukin.

5.4. Case Reports

Case Report 1

In July 2008, a 58-year-old patient presented at his family doctor with a pigmented interdigital skin lesion on his left foot, which he newly observed 1 month ago. Subsequently, the lesion was excised and a superficial spreading melanoma (SSM) with a Breslow thickness of 1.6 mm with no ulceration was diagnosed. The patient was referred to our department for a wide resection with 1 cm safety margins and sentinel lymph node biopsy (SNLB). The sentinel lymph node showed one micrometastasis (pT2apN1(sn1/1)M0). The tumor was BRAF and NRAS wild type.

An inguinal lymphadenectomy was performed in November 2008. The histology report revealed 15 tumor-free lymph nodes. In January 2009, the patient presented with, dark blue nodules highly suspicious of in-transit and satellite metastasis next to lymphadenectomy scar. LDH- as well as S100-levels were considerably increased. The PET-CT scans (Fig. 11) showed metastatic disease with diffuse hepatic, a small bowl metastasis in the right lower abdomen and multiple splenic metastasis as well as one solitary bone metastasis in the thoracic vertebra 6. The patient was included in the Sora-study (NCT00794235), an investigator controlled pilot study of sorafenib (multi kinase inhibitor) and dacarbazin (cytostatic) and was treated within the study protocol for 53 days. Unfortunately he progressed and died 2.4 months after the diagnosis of the distant metastasis at the age of 59.

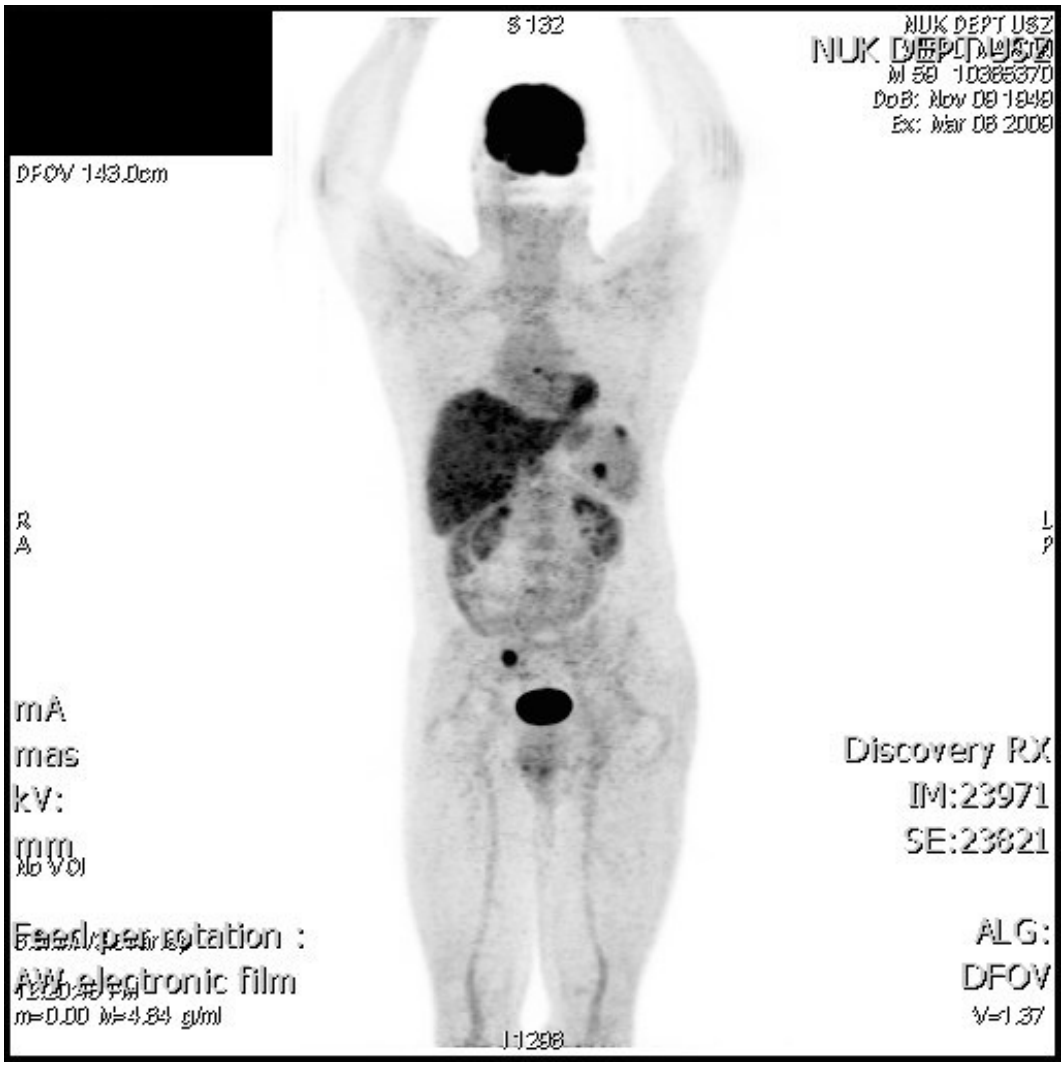

Fig. 11: PET-CT at stage IV of disease.

Case Report 2

A 42-year-old patient observed a non-pigmented dermal papule on his left thigh in spring 2012. The lesion was initially interpreted as benign by a regional dermatologist. Three months later and after a minimal trauma, the lesion started to bleed which concerned the patient. He then presented at his family doctor, who excised the lesion with CO2-laser. The histology showed an ulcerative, nodular malignant melanoma with a Breslow thickness of 2.17 mm. The patient was referred to our department for the further management and underwent a wide resection with 2cm safety margins and a SLNB. Upon positive SLNB, a left inguinal lymphadenectomy took place, which was clear. The patient harbored a BRAF mutation. He refused adjuvant immunotherapy with interferon due to possible adverse effects and was followed up according to high-risk protocol.

PET-CT scans in January 2013 indicated suspect ileocolic enhancements. A colonoscopy showed polyps without malignancy. Unfortunately the patient progressed 4 months later with multiple lymph node metastasis (retroperitoneal, para-aortic, next to the right musculus psoas, iliac internal and external (Fig. 12)). The bone-marrow puncture, which was initiated due to thrombocytopenia, revealed malignant infiltration. A therapy with vemurafenib (BRAF inhibitor) was initiated. Unfortunately the patient progressed and died two weeks after the diagnosis of a distant metastasis at the age of 53.

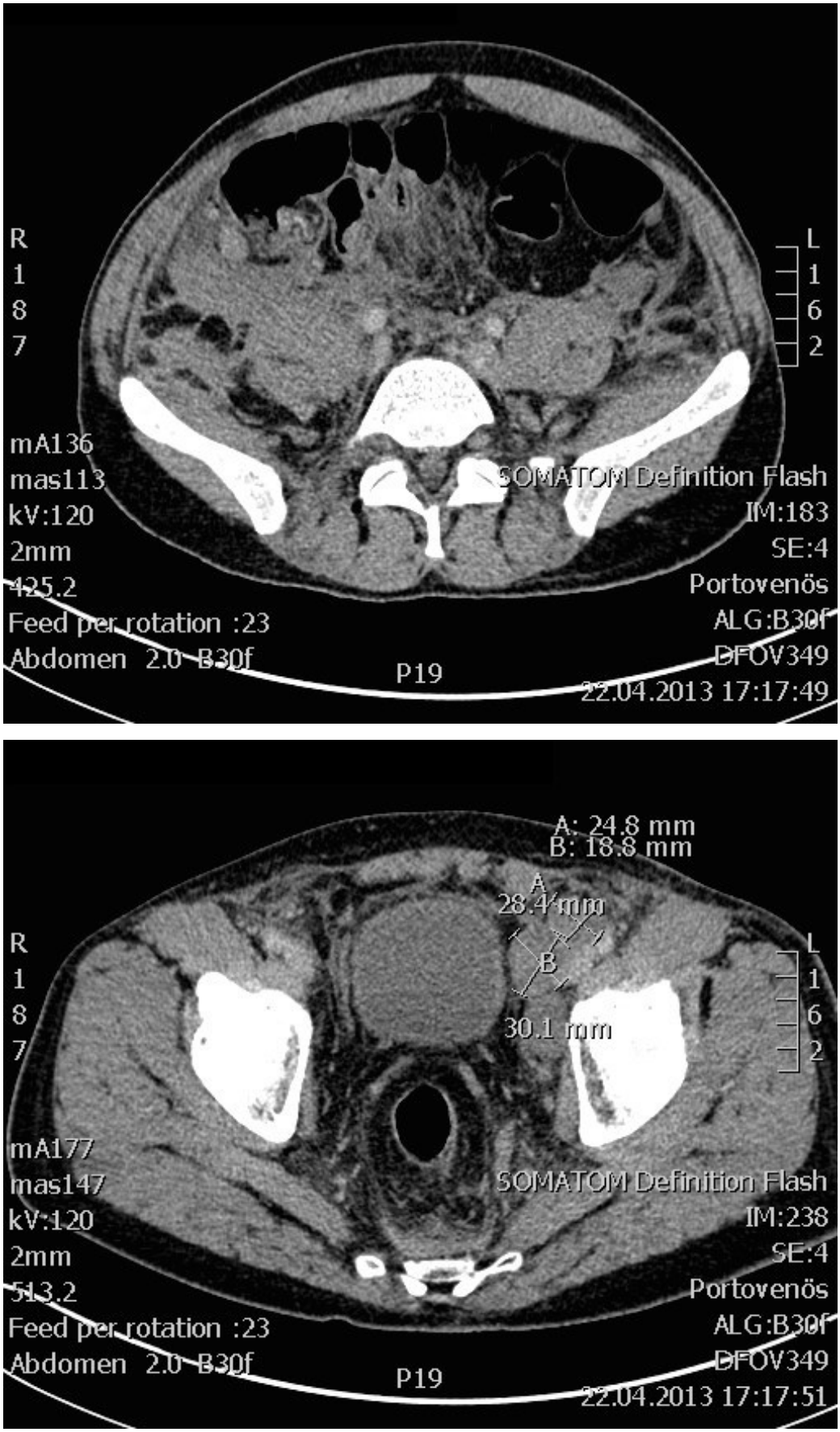

Fig. 12: CT scans at Stage IV of disease. Above: free liquid in the pelvis. Below: multiple lymph node metastasis.

Case Report 3

A 52-year-old patient with a past history of primary melanoma on her right upper arm completely excised in 2007, presented in February 2010 at her dermatologist, due to a hurtful scar at the site of the primary excision. Initially the scar was diagnosed as a keloid. The reddish firm lesion was highly suspicious of relapse, so the patient was referred to our department for resection, further treatment evaluation and staging. Histology showed a nodular malignant melanoma with a Breslow Index of 5.5mm. In-transit metastasis was also discussed in the differential diagnosis. Upon positive SLNB, the patient underwent an axillar lymphadenectomy, which showed additionally another metastatic lymph node (pT4N2M0). Gene analysis showed a N-RAS mutation.

Six months later, she progressed with a new liver metastasis (Fig. 13). At that time point, no further organs were affected. As the patient was B-RAF wild type, an inclusion in the Roche BRIM-3 study (NCT01006980) was not possible, so she was put on dacarbazin (alkylating chemotherapeutic agent). The patient completed 13 cycles of mono-chemotherapy, and due to progression of the mediastinal lymph node metastasis an additional therapy with sorafenib (multi kinase inhibitor) was implemented. Shortly after start, the patient developed a maculopapulous drug exanthema, so the treatment was interrupted.

CT-scan showed again disease progression in November 2011, with three new liver metastasis in segments II/V/VIII and progressive lymph node metastasis aortopulmonary, infracarinary and hilar. One month later, she started on ipilimumab with a mixed response after 4 cycles. She was then included in the Novartis-CEMK162X2102 clinical trial (NCT01352273), an open 1b phase study, with a combination of BRAF (RAF265) and MEK (MEK162) inhibitor (July 2012). Unfortunately and shortly after treatment initiation, the therapy was stopped again due to PD. A palliative symptomatic irradiation of the mediastinal lymph nodes with 39 Gray took place in October 2012. Due to further disease progression, she was treated with pembrolizumab in the MERCK MK-3475 study (NCT01704287). After six cycles (from March to June 2013), therapy was stopped because of progressive disease. A therapy with imatinib (protein kinase inhibitor) was implemented, again

without response. The patient died at the age of 55, 38.4 months after the diagnosis of a distant metastasis.

Fig. 13: PET-CT at stage IV of disease.

6. Discussion

The goal of our study was to analyze the group of patients with rapidly progressive lethal melanoma and to investigate their clinical characteristics and prognostic implications. To our knowledge, this is the first study to compare clinical characteristics of well-defined melanoma patients with short or long survival out of a large patient population (n=442).

LDH and S100 levels are already known to be important prognostic factors in metastatic melanoma disease (46, 47). We demonstrated that patients of our cohort with elevated LDH and/or S100 level are more likely to be in the short survivor group with OR=17 and OR=1.82, respectively (Fig. 7). We confirmed the negative impact of elevated LDH/S100 levels on the OS of these subpopulations.

Interestingly, more men are diagnosed with rapidly progressive melanoma, representing 69.6% of our SG. This finding correlates with Crocetti's recent statement, that female gender has a considerable protective effect on the mortality of malignant melanoma, though the reasons are still unknown (27). Possibly contributing to the women's better survival, is our speculation that men do not pay attention to their skin the same as women do and therefore go through fewer medical checkups at the dermatologist and present with more advanced disease.

It is to be expected that patients with NMM are more likely to be in the short survivor group, since the NMM has the most aggressive growth pattern and it is diagnosed rather late due to the missing radial growth phase (36). Nevertheless, both in our SG as well as in our CG cohort NMM was the most common subtype (30.4% and 20%), followed by SSM (21.7% and 16%). The most frequent localization of the primary melanoma was, in both groups, the lower limb. There is no significant difference regarding BRAF/NRAS mutations in-between the SG and CG (see Fig. 4/10).

Even though tumor thickness and ulceration status are strong prognostic factors and both part of the AJCC melanoma staging system (41), we do not have a significant higher rate of ulcerations or thick melanomas in our SG compared to the CG (see Table 1, Fig. 3/9). However, it has to be considered that these findings are biased by

the high quantity of unknown Breslow-index (34%) as well as ulceration status (68%) in the CG.

Our hypothesis, that patients with a direct hematogenous metastatic spread with no lymph node involvement are more likely to be part of the short survivor group could not be confirmed. There were too few patients with N0M1 stage in each group (SG: 8.7% versus CG: 4%), that none safe interpretation can be made. Nevertheless, the finding of Pleiss et al. speaks against our hypothesis. In this small study and among others, the 5-year survival of N1M0 versus N0M1 stage patients was investigated. They came to the result that N0M1 had better 5-year survival in comparison to N1M0 patients (61%, p=<0.02 versus 45%, p=<0.01) (53). As we expected, patients with a high number of afflicted organs were more likely to be in the SG than in the CG. There are clearly more patients with bone or liver metastasis, when entering stage IV disease, in the SG compared to the CG (shown in Tab. 1 and Fig. 8). Patients with brain metastasis were only in the SG. Herewith, we could verify bone, liver and brain metastasis as poor prognostic factors. Our assumption, that patients with lymph node and/or lung metastasis (= M1a/M1b) have a better prognosis than those with other visceral metastasis could also be confirmed. However, this result may be biased by the fact that patients with an elevated LDH level are counted in the M1c group besides the localization of their metastasis.

Clear limitations to our study are the retrospective setting as well as the small number of patients in the CG and especially the SG.

In conclusion, we identified a patient group with rapidly progressive melanoma in our institute. Based on the available clinical information we could confirm the negative prognostic factors such as elevated LDH and S100 levels, the male gender as well as the presence of bone, brain and liver metastasis at stage IV disease. Further immunohistochemical and molecular investigation is needed to fully understand the parameters, genetic or not, which implicate the rapidly progressive melanoma patients, as it seems that despite the new treatments there are still some patients, who die rapidly on their disease.

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8. Curriculum Vitae

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2011 – 2014	Studium der Humanmedizin an der Universität Fribourg
2014 – vorauss. 2017	Studium der Humanmedizin Universität Zürich

9. Declaration

Masterarbeit

Ich erkläre ausdrücklich, dass es sich bei der von mir im Rahmen des Studiengangs Master of Medicine (M Med) eingereichten schriftlichen Arbeit mit dem Titel

Characteristics and Prognostic Implications of rapidly progressive lethal Melanomas

um eine von mir selbst und ohne unerlaubte Beihilfe sowie *in eigenen Worten* verfasste Masterarbeit* handelt.
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Sanktionen
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Ich bestätige mit meiner Unterschrift die Richtigkeit dieser Angaben.

Datum:

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Unterschrift:……..

* Falls die Masterarbeit eine Publikation enthält, bei der ich Erst- oder Koautor/-in bin, wird meine eigene Arbeitsleistung im Begleittext detailliert und strukturiert beschrieben.